Functional and Nutritional Properties of Red Salmon (*Oncorhynchus nerka*) Enzymatic Hydrolysates

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ABSTRACT: The effects of different proteolytic enzymes and different reaction durations (25, 50, 75 min) on functional and nutritional properties of red (sockeye) salmon head hydrolysates were evaluated. Degree of hydrolysis values for the 75-min digestion ranged from 6.4% to 16.7%. Oil yield (4.9% to 10.6 %) from red salmon heads was affected by the enzyme used. Protein hydrolysate powders were yellowish and contained 62.3% to 64.8% protein with high levels of essential amino acids. Increased degree of hydrolysis values were weakly correlated with increased hydrolysate solubility. Maximum emulsion stability and fat adsorption were observed for the dried hydrolysate generated in the 25-min reaction time. Water adsorption of hydrolysate powders ranged from 1.0 mL to 3.3 mL water/g dried hydrolysate.

Keywords: fish hydrolysates, fish byproducts, functional properties, red salmon

Introduction

Fish processing byproducts including heads, frames, and viscera are often used for the production of fish meals and oils for the feed industry. Alaska produces more than 65% of the total wild fish harvested for human consumption in the United States. The annual harvest of Pacific salmon (*Oncorhynchus* spp.) from Alaska waters is nearly 300000 mt (ADFG 2003). Major byproducts from salmon processing include approximately 50000 mt of heads and 30000 mt of viscera. More than half of fish processing byproducts, especially those from sea processors, are not used (Crapo and Bechtel 2003). Alaska fish byproducts are good sources of high-quality proteins that can be further used as protein ingredients for food and feed applications (Bechtel and Johnson 2004; Sathivel and others 2004).

Fish-based protein powders have been commercially produced to enhance water-binding and frozen-stability properties of frozen seafood products (Urch 2001). Phillips and others (1994) reported that protein-rich seafood byproducts have a range of dynamic properties and can potentially be used in foods as binders, emulsifiers, and gelling agents. Proteins extracted from fish-processing byproducts can be modified to improve their quality and functional characteristics by enzymatic hydrolysis (Shahidi 1994). Fish protein hydrolysates (FPHs) having new and/or improved properties can be prepared using proteolytic enzymes; however, FPH properties can vary with the enzyme used and the reaction duration and conditions.

Hydrolyzed salmon protein is currently being produced in Alaska for use as an organic fertilizer as well as a pet and animal feed ingredient. However, the potential exists for the development of fish hydrolysate powders that can be incorporated into foods for human consumption. The objective of this study was to develop and characterize some properties of FPHs from red (sockeye) salmon heads that may be used as functional ingredients and nutritional supplements in human foods.

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Materials and Methods

Materials

Fresh red (sockeye) salmon (*Oncorhynchus nerka*) heads were obtained in early July from a commercial fish processing plant in Kodiak, Alaska. Fish heads were mechanically cut from the fish body and flumed in cold water on a byproduct line where samples were collected and immediately transported to the Fishery Industrial Technology Center within 15 min. The fish heads were immediately vacuum-packaged and stored at –40 °C until further processed. The commercial proteolytic enzymes Alcalase, Flavouryme 500L, Palatase 2000 L, and Neutrase were obtained from the Novo Nordisk, (Franklinton, N.C., U.S.A.) and Protex 6L and GC 106 were obtained from Genencor Intl., Inc. (Rochester, N.Y., U.S.A.).

Degree of hydrolysis (DH) and time course studies

A pilot study was performed in triplicate to determine the effect of heat treatment on the endogenous enzymes in red salmon heads. Red salmon heads were thawed at 4 °C overnight and minced in a Hobart mincer (K5SS, Hobart Corp., Troy, Ohio, U.S.A.). The head mince (50 g) was heated to 85 °C for 45 min in a water bath, then 50 g of water was mixed with the mince, and the temperature was adjusted to 50 °C. Comparison of %DH between nonheated and heated red salmon head minces was done in the presence or absence of 0.5% w/w Alcalase at both 0 and 50 min hydrolysis time.

The DH was measured using the method of Hoyle and Merritt (1994). At the end of each reaction time of 0 and 50 min, an aliquot (50 mL) was removed, mixed with 50 mL of 20% trichloroacetic acid (TCA) to obtain 10% TCA-soluble nitrogen and 10% TCA-insoluble nitrogen, and then centrifuged at 2560 × *g* for 15 min. The supernatant was decanted and analyzed for nitrogen content by a combustion method using the Leco FP-2000 nitrogen analyzer (LECO Corp., Mich., U.S.A.) that was calibrated with ethylenediaminetetraacetic acid as outlined in the Leco FP-2000 nitrogen analyzer manual. The

DH was calculated as [(10% TCA-soluble nitrogen in the sample) × 100]/Total nitrogen in the sample.

Three separate experiments were performed to determine the effect of hydrolysis time on DH using the method of Hoyle and Merritt (1994). Water (50 g) was added to a 50-g portion of red salmon head mince, and the mixture was heated to 50 °C. The Alcalase, Flavourzyme, Protex, Palatase 2000 L, GC 106, and Neutrase enzymes were added separately to the mince at 0.5% w/w. At the end of each hydrolysis time of 0, 15, 30, 45, 60, and/or 75 min, an aliquot (50 mL) was removed and determined for DH as explained previously.

Preparation of fish protein hydrolysate (FPH)

Red salmon heads were thawed at 4 °C overnight and minced in a Hobart mincer (K5SS, Hobart Corp.). The hydrolysis conditions were similar to those of Liceaga-Gesualdo and Li-Chan (1999) and Hoyle and Merritt (1994). The mince (500 g) was mixed with distilled water (500 g) and homogenized in a Waring blender for 2 min. The mixture was brought to 50 °C with constant stirring, and enzyme was added to the mince (0.5 g enzymes per 100 g head mince protein). Samples were continuously stirred at 50 °C and the enzyme for each mixture was inactivated at the end of each hydrolysis time of 25, 50, and 75 min by increased temperature above 85 °C for 15 min. The heated suspension was centrifuged at 2560 × *g* for 15 min, resulting in 3 separate phases: a semisolid phase at the bottom containing insoluble protein, bone, and skin; a heavy liquid phase in the middle containing soluble proteins; and a light liquid phase at the top containing the lipid fraction. The lipid layer was removed by aspiration, and then the semisolid phase and heavy liquid middle layers were removed together and freeze-dried. The resulting freeze-dried protein hydrolysates were placed in vacuum bags and stored at 4 °C until analyzed. The lipid yield (%) was calculated as [weight of lipid (g) × 100]/wet weight (g) of raw material.

Proximate composition

The red salmon head hydrolysate samples were analyzed in triplicate for moisture and ash contents using AOAC standard methods 930.15 and 942.05, respectively (AOAC 1995). Nitrogen content was determined in triplicate using the Leco FP-2000 Nitrogen Analyzer, and protein content was calculated as percent nitrogen × 6.25. The lipid content was determined using dichloromethyl ether as a solvent on an automated ASE-200 fat extractor (Dionex Corp., Sunnyvale, Calif., U.S.A.).

Color and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Color of red salmon head hydrolysate samples was determined in triplicate using the Minolta Chromameter (Model CR-300, Minolta Co., Ltd, Osaka, Japan) and reported as L^*, a^*, and b^* values. The SDS tricine/polyacrylamide gel electrophoresis system (Owl Separation Systems, Portsmouth, N.H., U.S.A.) was used to demonstrate chemical changes to protein bands with a Photodyne Foto/Force 300 (Hartland, Wis., U.S.A.) apparatus under reducing conditions according to Schagger and Von Jagow (1987). Novex Precast 10% to 20% gradient tricine gels (Invitrogen Life Technologies, Carlsbad, Calif., U.S.A.) were used, and molecular weight standards were purchased from Sigma-Aldrich (Number C 4105, St. Louis, Mo., U.S.A.). The protein bands were visualized from the gels stained with coomassie blue.

Amino acid and mineral analysis

Amino acid profiles were determined by the AAA Service Laboratory Inc., (Boring, Oreg., U.S.A.). Samples were hydrolyzed with 6 *N* HCl and 2% phenol at 110 °C for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with post column ninhydrin derivatization. Tryptophan and cysteine content were not determined.

Samples for mineral analysis were ashed overnight at 550 °C. Ashing residues were digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. Digested solutions were diluted and analyzed for As, Ag, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, Pb, Sr, and Zn by inductively coupled plasma optical emission spectroscopy on a Perkin Elmer Optima 3000 Radial ICP-OES (Perkin Elmer Life and Analytical Sciences, Inc., Boston, Mass., U.S.A.).

Functional properties

Functional properties for all red salmon hydrolysates were determined in triplicate. Nitrogen solubility was determined following the procedure of Morr and others (1985). Hydrolysate samples (500 mg) were dispersed in 50 mL of 0.1 *M* NaCl at pH 7.0, and the solution was stirred for 1 h at 25 °C and then centrifuged at 2560 × *g* for 30 min. The supernatant was analyzed for nitrogen content using a Leco FP-2000 Nitrogen Analyzer. Percent nitrogen solubility (%) was calculated as (supernatant nitrogen content × 100)/total nitrogen content of the sample.

Emulsifying stability (ES) was evaluated according to the method of Yatsumatsu and others (1972). Each red salmon hydrolysate sample (500 mg) was transferred into a 250-mL beaker and dissolved in 50 mL of 0.1 *M* NaCl, and then 50 mL of pure soybean oil (Hunt-Wesson Inc., Fullerton, Calif., U.S.A.) was added. A homogenizer equipped with a motorized stirrer (model 6-105-AF, Virtis Co, Gardner, N.Y., U.S.A.) driven by the rheostat was immersed in the mixture and operated for 2 min at 100% output (120 V) to make the emulsion. From the emulsion, 3 25-mL portions were immediately taken and transferred into 3 25-mL graduated cylinders. The emulsion samples were allowed to stand for 15 min at 25 °C, and then the aqueous volume and total volume were read. ES (%) was calculated as [(total volume – aqueous volume)/total volume] × 100.

The fat adsorption capacity of the red salmon hydrolysates was determined by placing 500 mg of each sample into a 50-mL centrifuge tube and adding 10 mL of soybean oil (Shahidi and others 1995). The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C with intermittent mixing every 10 min, and then centrifuged at 2560 × *g* for 25 min. Free oil was then decanted and the fat adsorption of the sample determined from the weight difference. Fat adsorption capacity was expressed as milliliters of fat adsorbed by 1 g of protein in red salmon hydrolysate powder. Water adsorption capacity of the red salmon hydrolysates was determined by placing 500 mg of each sample into a 50-mL centrifuge tube and adding 10 mL of distilled water. The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C with intermittent mixing every 10 min, then centrifuged at 2560 × *g* for 25 min. Free water was then decanted and the water adsorption of the sample determined from the weight difference. Water adsorption capacity was expressed as milliliters of water adsorbed by 1 g of protein in red salmon hydrolysate powder.

Statistical analysis

Statistical significance of observed differences among means of experimental results was evaluated by analysis of variance (ANOVA) (SAS Version 8.2, SAS Inst. Inc., Cary, N.C., U.S.A), followed by the post hoc Tukey's studentized range test (SAS Inst. 2002). Comparison of %DH between nonheated and heated red salmon head minces was performed using the 2-sample dependent *t* test (SAS Inst. 2002).

Results and Discussion

Degree of hydrolysis

Regardless of the hydrolysis time, DH of heated and nonheated

Table 1—Degree of hydrolysis (%DH) of red salmon heads as affected by heat and enzyme treatments[a]

Hydrolysis time (min)	Alcalase	Nonheated	Heated	Pr > | t |[b]
0	None	1.0 ± 0.3	2.4 ± 1.2	0.2
50	None	1.4 ± 0.4	1.6 ± 1.2	0.7
0	Yes	1.1 ± 0.2	3.5 ± 0.3	0.09
50	Yes	10.5 ± 0.9	8.8 ± 1.1	0.6

[a]Values are means ± SD of triplicate determinations.
[b]For each row, paired comparison of %DH was done between nonheated and heated samples.

Table 3—Effects of different enzymes and hydrolysis time on oil recovery (%) from red salmon heads[a]

	Hydrolysis time		
Enzymes	25 min	50 min	75 min
Alcalase	8.2 ± 0.4^{ab}	7.3 ± 1.6^{b}	10.6 ± 0.4^{a}
Flavourzyme 500L	7.7 ± 0.7^{a}	5.9 ± 1.1^{a}	8.1 ± 0.5^{a}
Palatase 2000L	7.3 ± 0.7^{a}	8.8 ± 0.2^{a}	8.5 ± 0.5^{a}
Protex 6L	8.8 ± 0.5^{a}	10.2 ± 1.0^{a}	8.4 ± 0.7^{a}
GC 106	8.4 ± 0.1^{a}	8.3 ± 0.5^{a}	4.9 ± 0.2^{b}
Neutrase	9.0 ± 0.7^{a}	8.9 ± 1.0^{a}	7.9 ± 0.3^{a}

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each row are not significantly different ($P > 0.05$).

Table 2—Proximate composition (%) of red salmon head mince and freeze-dried salmon head protein hydrolysates prepared with various enzymes[a]

Samples and enzymes	Protein	Lipid	Moisture	Ash
Red Salmon head mince	11.9 ± 0.7	14.5 ± 2.0	69.6 ± 2.7	4.0 ± 0.3
Alcalase	63.3 ± 2.1^{a}	23.7 ± 2.2^{a}	5.9 ± 0.4^{ab}	7.1 ± 0.5^{a}
Flavourzyme 500L	62.8 ± 0.4^{a}	24.5 ± 0.6^{a}	5.0 ± 0.1^{b}	7.7 ± 0.2^{a}
Palatase 2000L	62.3 ± 0.9^{a}	23.9 ± 1.4^{a}	6.1 ± 0.3^{a}	7.7 ± 0.4^{a}
Protex 6L	63.6 ± 0.7^{a}	23.1 ± 0.6^{a}	6.2 ± 0.4^{a}	7.1 ± 0.1^{a}
GC 106	64.8 ± 0.3^{a}	22.6 ± 0.5^{a}	5.6 ± 0.3^{ab}	7.2 ± 0.1^{a}
Neutrase	64.8 ± 4.7^{a}	22.5 ± 5.7^{a}	5.7 ± 0.5^{ab}	6.9 ± 0.6^{a}

[a]Values are means ± SD of triplicate determinations. Means (excluding the values from red salmon head mince) with the same letters in each column are not significantly different ($P > 0.05$).

samples was not statistically different ($P > 0.05$) both in the presence and absence of Alcalase enzyme (Table 1). At a 50-min hydrolysis time, the addition of the proteolytic enzyme Alcalase increased %DH for both heated (8.8 versus 1.6) and nonheated (10.5 versus 1.4) samples (Table 1). In this experiment, the exogenous enzyme present in the heads did not increase the DH values in the nonheated samples indicating low endogenous proteolytic activity.

For all enzymes, a steady increase in %DH was observed with increased hydrolysis time (Figure 1). Samples with Neutrase had the highest %DH at 15, 30, 45, and 60 min of hydrolysis time. However, the samples treated with Alcalase had the highest overall %DH at 75 min of hydrolysis. The lowest %DH was observed for samples treated with the GC 106 enzyme. After an initial rapid onset phase, the rates of hydrolysis tended to decrease, an observation consistent with that of Mackie (1982). The shape of hydrolysis curves (Figure 1) was similar to those previously published for other fish protein hydrolysates (Hoyle and Merritt 1994; Liceaga-Gesualdo and Li-Chan 1999; Sathivel and others 2003).

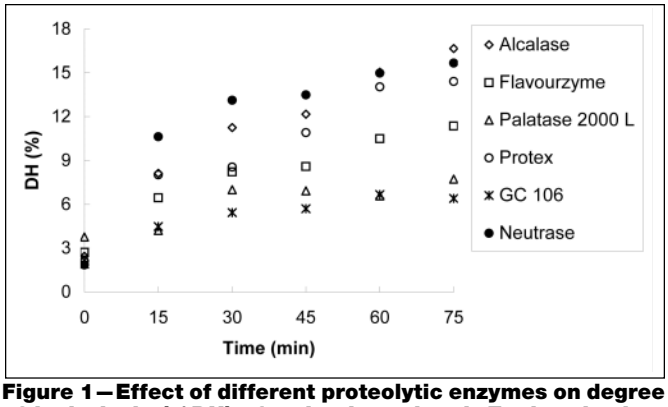

Figure 1—Effect of different proteolytic enzymes on degree of hydrolysis (%DH) of red salmon head. Each point is a mean of triplicate determinations.

Proximate composition and oil recovery

The red salmon head mince contained 69.6% moisture, 11.9% protein, and 4% ash (Table 2). The protein content of dried hydrolysates prepared with 6 commercial enzymes ranged from 62.3% to 64.8%, which was lower than those reported for herring protein hydrolysates (77% to 87.9%) by Sathivel and others (2003), Liceaga-Gesualdo and Li-Chan (1999), and Hoyle and Merritt (1994), and for Atlantic salmon protein hydrolysates (72% to 88%) by Kristinsson and Rasco (2000).

Red salmon head mince contained 14.5% lipid. The lipid content of red salmon hydrolysates ranged from 22.5% to 24.5%, which was higher than that reported for herring hydrolysates (1.5%) by Sathivel and others (2003) and for Atlantic salmon hydrolysates (0.23%) by Kristinsson and Rasco (2000). Sathivel and others (2005) reported that the lipid content of dried insoluble fish protein powder was significantly higher than that of dried soluble fish protein powder. In this study, soluble and insoluble protein fractions of the red salmon hydrolysates were, however, not separated. The ash content of red salmon hydrolysate samples ranged from 6.9% to 7.7%. Other investigators have reported fish protein hydrolysates with the ash content of 9% to 22% (Onodenalore and Shahidi 1996; Benjakul and Morrissey 1997; Liceaga-Gesualdo and Li-Chan 1999).

The crude oil recovery from the red salmon heads during hydrolysis ranged from 4.9% to 10.6% (Table 3). Increased oil recovery is expected with an increase in hydrolysis time; however, in this study there was no significant difference (except for Alcalase-treated samples) in the oil recovery with increased hydrolysis time. At 75 min hydrolysis time, alcalase treated red salmon heads had a higher oil recovery (10.6%) than other samples.

Color

The red salmon hydrolysates were dark yellow in color (Table 4). No differences in L^* color values (40.1 to 43.3) were observed among the red salmon hydrolysates. The GC 106 hydrolysate samples had ($P > 0.05$) the least yellow and red color with the b^* value of 22.3 and the a^* value of 8.1. The hydrolysates produced from

Table 4—Color *L*a*b values of red salmon head hydrolysates prepared with various enzymes at a 75-min hydrolysis time[a]**

Enzymes	*L**	*a**	*b**
Alcalase	43.3 ± 4.2a	9.0 ± 1.4ab	30.0 ± 1.4a
Flavourzyme 500L	42.2 ± 1.7a	9.9 ± 0.7a	30.2 ± 1.6a
Palatase 2000L	40.8 ± 2.3a	10.0 ± 0.5a	29.6 ± 0.5ab
Protex 6L	40.1 ± 4.0a	10.2 ± 0.7a	30.8 ± 1.3a
GC 106	42.6 ± 0.7a	8.1 ± 0.4b	22.3 ± 0.6c
Neutrase	43.0 ± 1.4a	9.9 ± 0.2a	27.9 ± 0.5b

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each column are not significantly different ($P > 0.05$).

this study had greater yellowness and redness than those reported by Sathivel and others (2003) for herring hydrolysates (b^* = 8 to 18 and a^* = 2.8 to 4.2). More yellowness and redness of the red salmon hydrolysates may be due to greater retention of lipids and lipid soluble pigments.

Amino acid and mineral analysis

The essential amino acid contents (milligrams of amino acid/gram protein) of all samples were higher than the recommended values for human adults (FAO/WHO 1990) (Table 5). All red salmon hydrolysates exceeded the lysine requirements for infants (FAO/WHO/UNU 1985). The lysine content of red salmon hydrolysates ranged from 71.4 to 83.2 (milligrams of amino acid per gram protein) and was similar to that (80.4 mg of amino acid per gram protein) reported for herring head hydrolysate (Sathivel and others 2003). The content of many of the essential amino acids exceeded the requirements for infants (FAO/WHO/UNU 1985).

All red salmon hydrolysates were rich in K, P, Ca, and Mg as well as micronutrients including Zn and Fe (Table 6). The Ca to P ratios ranged from 0.81 to 1.9. The contents of heavy metal including Pb and Sr, Cd were low.

Functional properties

Functional properties influence the usefulness of an ingredient in food and govern the physical behavior during preparation, processing, and storage (Fennema 1996). Solubility is one of the most important properties of proteins (Kinsella 1976; Mahmoud and others 1992). Nitrogen solubility values for the red salmon hydrolysates are shown in Table 7. The nitrogen solubility values of the red salmon hydrolysates ranged from 17.2% to 54.4%, which were lower than the values reported by Sathivel and others (2004) for the soluble fraction of herring hydrolysates (84.9%). The high solubility of red salmon hydrolysates prepared with Alcalase and Protex indicates potential application in formulated food systems by providing improved appearance and smooth mouthfeel to the product (Petersen 1981).

Increased solubility is expected with increased hydrolysis time (Shahidi 1994); however, there were few significant differences in solubility as a function of hydrolysis times for each of the enzymes tested here (Table 7). High solubility of fish protein hydrolysates is often due to cleavage of proteins into smaller peptide units that usually have increased solubility (Shahidi 1994). Increased solubility is not only due to smaller peptide size but is also due to the balance of hydrophilic and hydrophobic elements in the peptides. The smaller peptides from myofibrillar protein are expected to have more polar residues, increasing hydrophilicity through an increased ability to form hydrogen bonds with water.

Emulsifying stability of red salmon head hydrolysates ranged from 66.9% to 100% under the conditions in this study (Table 8). Emulsifying stability values of 52% to 61.0% and 48.5% to 54.2% were reported for Atlantic salmon protein hydrolysates by Kristinsson and Rasco (2000) and for herring protein hydrolysates by Sathivel and others (2003), respectively. The emulsion of hydrolysates produced with Alcalase, Palatase 2000 L, and GC 106 at 75 min hydrolysis time was less stable than those produced from the same enzymes at 25 min. This may be due to the presence of smaller peptides, which are less effective in stabilizing emulsions (Quaglia and Orban 1990).

Figure 2 shows the electrophoretic profile of the 25, 50, and 75 min digestions of red salmon head protein with the 6 enzyme treatments. Only a few higher-molecular-weight protein bands after 25 min hydrolysis were found for Alcalase (Figure 2a, lane 1) and Protex (Figure 2a, lane 4) treatments. Treatments with greater numbers of high-molecular-weight bands after 25 min of hydrolysis were Palatase (Figure 2b, lane 4) and GC 106 (Figure 2c, lane 4). As can be

Table 5—Amino acid composition of red salmon head hydrolysates prepared with various enzymes at a 75-min hydrolysis time

Amino acids[a]	Alcalase	Flavourzyme	Palatase	Protex	GC 106	Neutrase
Hydroxyproline	23.5	25.6	26.6	23.4	12.4	17.0
Aspartic acid	88.3	87.7	87.2	89.3	94	91.1
Threonine[b]	41.9	42.1	42.6	43.9	46.3	44.0
Serine	46.1	46.2	46.4	46.1	45.7	44.2
Glutamic acid	135.1	134.9	133.3	132.5	138.5	135.6
Proline	65.0	66.8	66.6	64.8	53.1	58.2
Glycine	97.6	102.9	105.3	99.6	72.4	85.2
Alanine	68.4	68.8	69.6	69.2	66.3	67.7
Valine[b]	50.1	45.2	45.5	48	51.4	50.2
Methionine[b]	28.8	29.4	28.9	28.6	30.3	30.0
Isoleucine[b]	37.1	36.6	35.7	37.4	42.4	39.9
Leucine[b]	66.9	66.5	66.0	65.7	77.1	72.0
Tyrosine	32.5	30.9	31.0	32.1	36.2	34.8
Phenylalanine[b]	40.7	39.7	39.6	39.3	44.5	42.0
Histidine[b]	23.8	23.0	23.8	24.7	27.7	26.6
Lysine[b]	73.9	72.5	71.4	76.1	83.2	81.6
Arginine	67.7	69.2	68.4	69.2	66.8	69.6
TEAA[c]	363.2	355	353.5	363.7	402.9	386.3
TAA[c]	987.4	988	987.9	989.9	988.3	989.7
TEAA/TAA%	36.8	35.9	35.8	36.7	40.8	39.0

[a]Data expressed as mg of amino acid per gram protein. Tryptophan and cysteine were not determined.
[b]Essential amino acids for infants.
[c]TAA = total amino acids; TEAA = total essential amino acids for infants.

Table 6—Mineral content of red salmon head hydrolysates prepared with various enzymes at a 75-min hydrolysis time

Minerals	Alcalase	Flavourzyme	Palatase	Protex	GC 106	Neutrase
P (%)	0.47	0.55	0.57	0.58	0.59	0.54
K (%)	0.73	0.77	0.84	0.75	0.98	0.9
Ca (%)	0.43	0.68	0.7	0.7	0.31	0.31
Mg (%)	0.1	0.13	0.13	0.13	0.13	0.13
Cu (ppm)	35.8	30.7	35.5	38.5	104.7	102.6
Zn (ppm)	66.0	79.0	71.0	65.0	67.0	70.0
Mn (ppm)	<1	<1	<1	<1	<1	<1
Fe (ppm)	123	140	85.0	94.0	164	99.0
Cd (ppm)	0.19	0.13	0.26	0.23	0.54	0.36
Ni (ppm)	0.74	<0.20	<0.20	<0.20	0.9	<0.20
Pb (ppm)	3.51	1.32	1.57	2.21	1.5	<0.20
Ag (ppm)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Sr (ppm)	23.7	35.11	36.81	39.17	16.3	17.24
As (ppm)	2.5	1.6	0.7	0.3	4	2.9

Table 7—Nitrogen solubility (%) of red salmon head hydrolysates[a]

	Hydrolysis time		
Enzymes	25 min	50 min	75 min
Alcalase	54.4 ± 3.6[a]	47.3 ± 1.4[b]	53.9 ± 1.3[a]
Flavourzyme 500L	27.7 ± 4.2[b]	28.9 ± 2.4[b]	42.2 ± 4.2[a]
Palatase 2000L	23.3 ± 5.3[b]	26.2 ± 3.0[b]	39.4 ± 6.3[a]
Protex 6L	49.4 ± 1.2[a]	53.1 ± 6.5[a]	45.1 ± 4.2[a]
GC 106	17.2 ± 1.2[b]	36.4 ± 1.1[a]	19.1 ± 2.5[b]
Neutrase	39.6 ± 3.9[b]	54.1 ± 6.8[a]	41.7 ± 1.6[b]

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each row are not significantly different ($P > 0.05$).

Table 8—Emulsifying stability (%) of red salmon head hydrolysates[a]

	Hydrolysis time		
Enzymes	25 min	50 min	75 min
Alcalase	84.2 ± 5.0[a]	73.6 ± 0.8[b]	71.1 ± 0.8[b]
Flavourzyme 500L	100 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]
Palatase 2000L	77.9 ± 3.2[a]	82.4 ± 7.1[a]	66.9 ± 2.3[b]
Protex 6L	74.3 ± 3.6[a]	69.5 ± 4.5[a]	72.8 ± 1.3[a]
GC 106	99.9 ± 0.1[a]	69.6 ± 5.6[b]	72.1 ± 1.2[b]
Neutrase	74 ± 2.2[a]	76.4 ± 1.0[a]	74.3 ± 2.2[a]

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each row are not significantly different ($P > 0.05$).

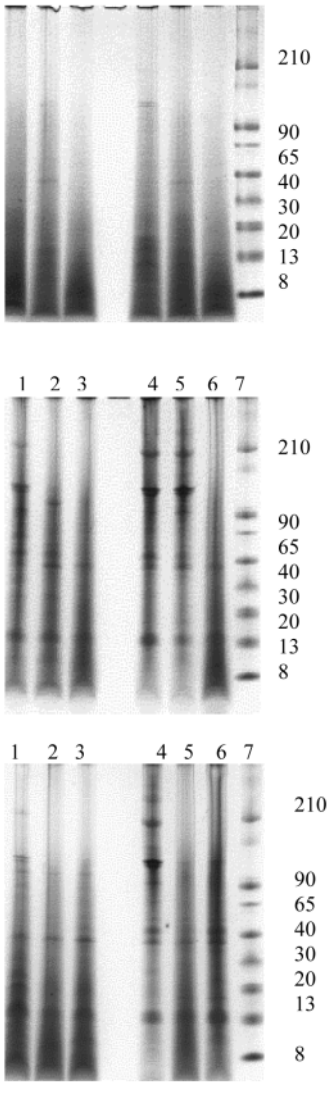

Figure 2—Electrophoresis profiles of the salmon protein hydrolysates and the sodium dodecyl sulfate (SDS) marker. (a) for Alcalase at 25 min (lane 1), 50 min (lane 2), 75 min (lane 3), and for Protex at 25 min (lane 4), 50 min (lane 5), 75 min (lane 6), and the SDS marker (8 to 210 kDa, lane 7); (b) for Flavourzyme at 25 min (lane 1), 50 min (lane 2), 75 min (lane 3), and for Palatase at 25 min (lane 4), 50 min (lane 5), 75 min (lane 6), and the SDS marker (8 to 210 kDa, lane 7); (c) for Neutrase at 25 min (lane 1), 50 min (lane 2), 75 min (lane 3), and for GC 106 at 25 min (lane 4), 50 min (lane 5), 75 min (lane 6), and the SDS marker (8 to 210 kDa, lane 7).

seen from Figure 2, the 25-min digestion resulted in less degradation of higher-molecular-weight proteins when compared with either the 50- or 75-min digestions. From Figure 2, we can generalize that there is an increased loss of higher-molecular-weight proteins with increased time of digestion. This is an observation consistent with the increased DH values shown in Figure 1.

The emulsion stability for hydrolysates produced with alcalase and GC 106 was greater at 25 min DH than at 50 min and 75 min DH. Gauthier and others (1993) and Jost and others (1977) examined the role of peptide characteristics on emulsification properties. They reported that hydrophobicity and peptide lengths influenced the emulsifying properties. Peptides often have reduced emulsifying properties (Chobert and others 1988). A positive correlation between surface activity and peptide length was reported (Jost and others 1977), and a peptide should have a minimum length of 20 residues to possess good emulsifying and interfacial properties (Lee and others 1987). A clear relationship between the peptide size (Figure 2) and emulsion stability (Table 8) for hydrolysates produced from Flavourzyme, Protex, and Neutrase was not evident, indicating that physicochemical properties of peptides might play an important role in the difference observed in the emulsion stability.

Fat-binding capacity (fat adsorption) is an important functional characteristic of food ingredients used in the meat and confectionary industries (Shahidi and others 1995). The fat adsorption of red salmon head hydrolysates ranged from 3.7 to 7.8 mL oil/g protein (Table 9). Other reports of fat adsorption capacity values include values from 3.9 to 11.5 mL of oil/g protein for herring protein powders (Sathivel and others 2004), 3.7 to 7.3 mL of oil/g protein for hydrolyzed herring byproduct proteins (Sathivel and others 2003), and 2.86 to 7.07 mL of oil/g protein for Atlantic salmon protein hydrolysates (Kristinsson and Rasco 2000).

Red salmon hydrolysates produced at a 25-min hydrolysis time had a significantly greater ability to bind soybean oil than hydrolysates produced from the same enzymes at 75 min, except for the GC 106 treatment. The mechanism of fat-binding capacity is mainly due to physical entrapment of the oil. Also protein powders with higher bulk density bind greater amounts of fat (Kinsella 1976); however, the bulk density of the samples was not determined in

Table 9—Fat-adsorption capacity of red salmon head hydrolysates[a]

Enzymes	Hydrolysis time		
	25 min	50 min	75 min
Alcalase	5.0 ± 0.4a	5.1 ± 0.4a	3.9 ± 0.3b
Flavourzyme 500L	7.4 ± 0.5a	6.2 ± 0.2b	4.1 ± 0.3c
Palatase 2000L	6.9 ± 0.6a	7.8 ± 0.8a	4.3 ± 0.1b
Protex 6L	5.7 ± 0.2a	6.0 ± 0.3a	3.7 ± 0.1b
GC 106	6.1 ± 0.3a	4.9 ± 0.1b	7.0 ± 0.4a
Neutrase	5.5 ± 0.1a	5.4 ± 0.3a	3.8 ± 0.1b

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each row are not significantly different ($P > 0.05$).

Table 10—Water-adsorption capacity of red salmon head hydrolysates[a]

Enzymes	Hydrolysis time		
	25 min	50 min	75 min
Alcalase	1.7 ± 0.7a	1.6 ± 0.2a	1.4 ± 0.3a
Flavourzyme 500L	2.0 ± 0.1b	2.7 ± 0.1a	2.3 ± 0.3ab
Palatase 2000L	3.1 ± 0.1a	2.6 ± 0.1b	2.6 ± 0.1b
Protex 6L	2.6 ± 1.2a	1.5 ± 0.3a	1.0 ± 0.2a
GC 106	3.3 ± 1.7a	2.9 ± 0.2a	3.1 ± 0.2a
Neutrase	1.7 ± 0.1a	1.7 ± 0.1a	1.7 ± 0.1a

[a]Values are means ± SD of triplicate determinations. Means with the same letters in each row are not significantly different ($P > 0.05$).

this study. The majority of the red salmon hydrolysates exhibited comparable or better fat adsorption capacity than that reported by Sathivel and others (2003) for egg albumin (5.1 mL of oil/g protein) and soy protein concentrate (3.6 mL of oil/g protein). These hydrolysates could potentially be used as functional ingredients for meat and confectionery industry.

Water adsorption refers to the ability of the protein to adsorb water and retain it against centrifugal force within a protein matrix (Fennema 1996). Several protein ingredients are used as water-holding additives in muscle foods; however, fish proteins are not widely used as water-adsorption agents. The ability of red salmon hydrolysates to adsorb water is given in Table 10. In general, the water-adsorption capacity was not affected by hydrolysis time, except for hydrolysates prepared with Palatase. However, there were some differences between treatments within a given hydrolysis time. The red salmon hydrolysate sample produced with Palatase at a 25-min hydrolysis time had a greater ability to bind water than hydrolysates produced from the same enzymes at 50 min and 75 min.

Conclusions

This study demonstrated that the degree of hydrolysis and resulting peptide properties were affected by both digestion time and the type of commercial enzyme used. Protein hydrolysates derived from red salmon heads are a good source of essential amino acids and minerals. Oil recovery from the salmon heads was somewhat affected by the kind of proteolytic enzymes used and the digestion time. The red salmon hydrolysate powders had desirable dark yellow color. Functional properties (solubility, fat adsorption, water adsorption, and emulsification stability) of the red salmon hydrolysates are consistent with potential applications as emulsifiers and binder agents; therefore, the red salmon hydrolysates could potentially compete with dairy-based and plant-based protein hydrolysates and protein powders currently available in the market place. This study identified opportunities to develop value-added products from Alaska salmon processing byproducts.

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